for thermogenic needs of animals. Therefore thermogenesis on the level of brown-fat mitochondria and whole animal was examined here in PolgA mtDNA polymerase mutant mice (Mutator) exhibiting numerous mutations of mtDNA and several features of premature aging (Trifunovic et al., 2004). As compared with wild-type mitochondria, on all 3 substrates investigated (pyruvate, palmitoyl-L-carnitine and glycerol-3-phosphate), UCP1-dependent oxygen consumption was significantly reduced in mutant mitochondria similarly to maximal oxidative capacity (FCCP-response), indicating impaired thermogenesis on the level of brown-fat mitochondria in Mutator mice. Basal metabolic rate at 30 °C (thermoneutrality) was higher in mtDNA-Mutator mice as compared with WT mice; this may indicate changed set-point of the thermoregulatory centre. However, cold-induced metabolic rate (estimated as increase in oxygen consumption at 22 °C compared to 30 °C) in Mutator mice was only half of that in WT. At environmental temperatures below 20 °C, Mutator mice were unable to further increase their metabolism and went into torpor. Response to adrenergic stimulus (NE injection) was significantly reduced in Mutator mice. Thus, mtDNA mutation led to lower activity of brown-fat mitochondria and impaired thermogenesis; i.e. also in this respect, mtDNA-Mutator mice mimicked normal ageing.

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S14.7 Impact of chronological aging on mitoproteome of *Saccharomyces cerevisiae*

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The free radical theory of aging postulates that reactive oxygen species (ROS) mainly produced by mitochondria are able to induce cellular damages leading to cell death. In *Saccharomyces cerevisiae*, chronological aging is related to the senescence over time of non dividing cells. In this work we study chronological aging of *S. cerevisiae* in stationary phase with a proteomic approach (Two Dimensional Differential in-gel electrophoresis methodology) in order to compare the mitochondrial proteome at three distinct periods (0 day, 7 days, and 14 days). Moreover, based on a recent study in stationary phase culture (Allen, 2006), we separated quiescent (Q) from non-quiescent (NQ) cells which mainly differ by their ability to form colonies on Petri dishes. Down-regulations of the major mitochondrial metabolic pathways (Krebs cycle, OXPHOS, amino-acid metabolism, protein synthesis, folding and import) were observed between 7 and 14 days. Interestingly, the only differential regulation observed between Q and NQ cells at 7 days is related to the ROS detoxifying enzyme glutathione transferase that was found to be more expressed in Q cells. This result suggests that Q cells mitochondria have a better capacity to resist to oxidative stress, and could partially explain why these latter cells are able to form colonies again.

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S14.8 Top-down control analysis of mitochondrial oxidative phosphorylation: From mitochondria to pathologies

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The top-down approach of the metabolic control analysis, extensively used in our laboratory, has for example been applied on isolated mitochondria to describe the protective role of cyclosporin A on mitochondrial function during ischemia–reperfusion transitions. In this approach oxidative phosphorylation is described as large modules linked by a common intermediate. In this system the respiratory chain generates the proton-motive force, which is consumed by the phosphorylation subsystem and proton leak across the inner mitochondrial membrane. By monitoring simultaneously the kinetics of oxidation and phosphorylation rates and the membrane potential variations, it becomes possible to determine the elasticity of those three modules in response to small variations of the proton-motive force (obtained experimentally over a range of phosphorylation rates from state 4 to 3) and thus to access to the control scheme of oxidative phosphorylation. We will present our first results obtained on rat skeletal muscle mitochondria, in which respiratory chain exerts an important control, whatever the phosphorylation rate. This approach will be used in a near future to investigate the effect of aging and septic shock on mitochondrial function. With the top-down control analysis, we will seek to determine which modules or processes are affected by these conditions and thus better understand the very mechanisms responsible of observed mitochondrial and muscle dysfunctions.

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S14.9 Testing the mitochondrial free radical theory of aging in *Drosophila melanogaster*

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Nowadays, the Mitochondrial Free Radical Theory of Aging (MFRTA) is the most supported theory to explain aging process. It is possible to deduce three predictions from MFRTA: 1) long-lived animals must produce fewer mitochondrial Reactive Oxygen Species (mtROS) than short-lived ones, 2) the decrease in mtROS generation must increase life span and 3) the increase in mtROS generation must decrease life span. In the present study we have used *Drosophila melanogaster* to test such predictions. First, we have study mtROS production in three different wild type strains of *Drosophila* (Dahomey, Canton-S and Oregon). According to MFRTA long-lived Oregon flies produce fewer mtROS than short-lived Dahomey or Canton-S. In order to test the second prediction we have introduce the alternative oxidase (AOX) gene from *Ciona intestinalis* in *Drosophila* genome. AOX expression decrease free radical production, but it does not increase mean or maximum life span at three different temperatures (18, 25 and 29 °C). We tested the third prediction in DJ-1 mutant flies. DJ-1 mutant flies produce significantly more free radicals than Oregon, Canton-S or Dahomey flies, however at 25 °C they do not live shorter than the longest-living background (Oregon). In summary, our results do not support MFRTA and they invite to re-think the role of mtROS in aging process.

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S14.10 Estimation of membrane potential of rat liver mitochondrial particles by TMRE fluorescence in confocal mode

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